Mastitis pathogen identification using polymerase chain reaction in New Zealand milk samples

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ABSTRACT

Rapid identification of the pathogen responsible for an intramammary infection in a dairy cow can support mastitis management decisions. Polymerase chain reaction (PCR) has become available to identify mastitis pathogens in milk, offering a rapid and sensitive test. The performance of a commercial, real-time PCR assay (PathoProof Complete-12 Mastitis PCR assay; Thermo Fisher Scientific Ltd., Vantaa, Finland) was compared with traditional bacterial culture for the identification of the most frequent pathogens in New Zealand, *Streptococcus uberis* and *Staphylococcus aureus*, during three stages of lactation. Aseptically collected quarter milk samples were analysed by culture and a subset (n=343) selected for PCR analysis based on infection status in culture. Using culture as the reference test, PCR had a relative sensitivity and specificity of 86.8%, and 87.7% (kappa=0.74) for detecting *S. uberis* and 96.4% and 99.7% (kappa = 0.96) for detecting *S. aureus*. Relative sensitivity for detecting *S. uberis* was similar throughout lactation whereas relative specificity was lower at the first milking post-calving (64%) and higher in mid-late lactation (97.7%). Initial validation of the PCR assay identified issues in *S. uberis* detection, particularly when milk samples were from freshly calved cows or from cows whose milk contained clots indicating clinical mastitis. Dilution of some colostrum and some clinical samples was required for detection of bacteria by PCR, due to the presence of PCR inhibitors in the milk. The PCR assay used in this study is not recommended for mastitis pathogen identification in early lactation as the majority of infections caused by *S. uberis* occur in the first month of lactation. PCR testing offers a number of opportunities and advantages to improve udder health and milk quality but for uptake in New Zealand, development is required to better suit colostrum samples. Greater clarity is required regarding the interpretation of PCR results and the use of information from such tests for decision-making.

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THESIS STRUCTURE

This thesis follows a traditional structure, beginning with an introduction to mastitis pathogen identification and a review of the literature in mastitis diagnostics, in particular the use of polymerase chain reaction (PCR) as an alternative to traditional bacterial culture to identify mastitis-causing pathogens. This develops the justification for the study objectives and hypothesis. The materials and methods section is split into two components, firstly considering milk sample collection, bacterial culture, and PCR testing. The second methods chapter comprises the procedures involved in the development of the PCR assay for use on New Zealand milk samples. Finally, the results are presented, discussed, and analysed.